Immunology 1996 **87** 213–219

Developmental heterogeneity of Vγ1.1 T cells in the mouse liver

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SUMMARY

Modifications at V–(D)–J junctions increase the diversity of T-cell receptors (TCR). It has been shown that the levels of N-nucleotide insertion at the V–(D)–J junction in TCR transcripts are different between fetal and adult stages. To clarify developmental stages and pathways of γδ T cells in the liver, we analysed the nucleotide sequence of Vγ1.1–Jγ4 junctions of intra-hepatic lymphocytes (IHL), spleen cells and developing thymocytes from normal and athymic nude mice. The level of N-insertion increased in thymocytes during ontogeny. The percentage of Vγ1.1–Jγ4 transcripts with N-insertion was 3% at day 16 of gestation, 42% at newborn, and 89% at 7 weeks. Transcripts from normal IHL showed intermediate levels of N-insertion between those of newborn and adult thymocytes. In contrast the percentage of N-insertion in nude IHL was 47%, and this value was comparable to that of newborn thymocytes. Among the transcripts of normal IHL, the sequences common with nude IHL showed a newborn level of N-insertion (38%), and the remaining sequences showed an adult level (89%). These results suggested the possibility that Vγ1.1-expressing T cells in IHL might be a heterogeneous population consisting of the cells developed extrathymically as well as the cells developed intrathymically. The Vγ1.1–Jγ4 junctions from spleen cells showed less variability than those from IHL and adult thymocytes. It suggested that γδ T cells bearing specific Vγ1.1 TCR develop and/or home in the spleen.

INTRODUCTION

V–(D)–J rearrangement generates junctional diversity of T-cell receptors (TCR).[1] N-nucleotide insertions, nibbling of coding ends, and P nucleotide additions at the junctions further increase the diversity.[2] We have reported previously that a developmental switch in Vγ3 T-cell differentiation occurs at the level of haematopoietic stem cells,[3,4] and that the capacity of N-insertions in Vγ4–Jγ1 junctions changes at the level of T precursors during ontogeny.[5]

The development of γδ T cells is highly programmed during ontogeny by the expression of different Vγ gene segments.[4,6] The γδ T cells expressing distinct Vγ genes are distributed in specific tissues, such as the skin, the reproductive tracts, the intestine, and the lymphoid organs.[7]

The murine liver is one of the anatomical sites where the γδ T cells reside.[8–10] The γδ T cells in the liver have been reported to appear with age-related kinetics, and are large blast cells in morphology, and show $CD4^-CD8^-$ or $CD4^-CD8\alpha\alpha^+$ surface phenotypes.[8] Most of heat-shock protein (HSP) 60-reactive γδ T-cell hybridomas established from intrahepatic lymphocytes (IHL) expressed Vγ1.1 TCR,[9] implying a functional role of the γδ T cells in the liver. Vγ1.1 T cells in the skin and the intestinal epithelium are reported to have developed through extrathymic pathways.[11,12] The γδ T cells in the liver are also considered to have developed extrathymically. However, their developmental stage is not clear.

To clarify the pathway and the stage of Vγ1.1 T-cell development in the liver, we analysed the nucleotide sequences of Vγ1.1–Jγ4 junctions from IHL and spleen cells of normal and athymic nude mice. Our results suggested the possibility that IHL might contain Vγ1.1 T cells developed extrathymically around the newborn stage, and that T cells bearing specific Vγ1.1 TCR might develop and/or home selectively in the spleen.

MATERIALS AND METHODS

Mice

Athymic nude and normal BALB/c mice of 7–8 weeks of age were purchased from Charles River Inc. (Atugi, Japan). Newborn mice and fetuses at day 16 of gestation were obtained by timed mating of BALB/c mice. The age of fetuses was determined by scoring for the appearance of a vaginal plug and taking as day 0 the morning on which the mating plug was observed. Mice were bred and maintained under specific

Received 22 June 1995; revised 22 September 1995; accepted 27 September 1995.

Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; HSP, heat-shock protein; IHL, intrahepatic lymphocytes; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

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pathogen-free conditions in the Animal Center for Bioresearch (Faculty of Medicine, University of Tokyo, Tokyo, Japan).

Cell preparations

Mice were anaesthetized with sodium pentobarbital (50 mg/kg body weight, intraperitoneally; Abbott Laboratories, North Chicago, IL), and then livers were perfused via the portal vein with 30 ml of Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (Gibco BRL, Gaithersburg, MD). Excised livers were minced and incubated in the solution containing 0·05% of collagenase (Wako Pure Chemical, Osaka, Japan) for 15 min under shaking at 37°. After passing through nylon mesh sheets, the single-cell suspension was centrifuged for 1 min at 30 *g* to pellet down hepatocytes. The cells in the supernatant were then passed over a nylon wool column to enrich T cells. The nylon wool non-adherent cells are referred to as IHL.[13] Spleen cells and thymocytes were isolated as described previously.[3]

Polymerase chain reaction (PCR)

Poly $(A)^+$ RNA was isolated from 1×10^6 cells and cDNA was synthesized as described previously.[3] cDNA were amplified with a reaction buffer containing 50 mM KCl, 10 mM Tris–HCl (pH 8·3), 1·5 mM $MgCl_2$, 0·001% (w/v) gelatin, 20 ng/ml of each primer of the appropriate pair, 0·2 mM of each deoxynucleotide triphosphate, and 25 U/ml of Taq DNA polymerase (Takara, Otsu, Japan). Each cycle consisted of denaturation (1 min at 94°), primer annealing (1 min at 50°), and extension (1 or 2 min at 72°) steps. The reactions were run for 25 cycles by a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). Primers used were terminal deoxynucleotidyl (TdT) 5′, 5′-ACTGCGACATCTTAGAGTCA-3′; TdT3′, 5′-CTTCCCCTTAGTCCTGTCAT-3′; hypoxanthine phosphoribosyl transferase (HPRT) 5′, 5′-CTCGAAGTGTTGGATACAGG-3′; and HPRT 3′, 5′-TGGCCTATAGGCTCATAGTG-3′. PCR products were electrophoresed through a 1% agarose gel and blotted to nylon membranes (Biodyne A; PALL, Biosupport, East Hills, NY). The membrane was hybridized with ^{32}P-labelled probes (280 bp fragment of TdT cDNA, or 350 bp fragment of HPRT cDNA) and the radioactivity was detected and quantified with an Bioimaging Analyzer (Fujix BAS2000; Fuji Film, Tokyo, Japan).

DNA sequencing

PCR was carried out for 40 cycles under the same conditions as described above with primers as follows: Vγ1.1, 5′-GGAAGCTTCTACAATCAACGACCCTTAGG-3′ and Cγ4, 5′-CCGAATTCGGAGAAAAGTCTGAGTCAGT-3′. The underlined sequences represent the restriction sites of *Hin*dIII and *Eco*RI, respectively. The amplified cDNA was digested with *Eco*RI and *Hin*dIII, gel purified with a MERmaid kit (Bio 101 Inc., La Jolla, CA), and cloned into Bluescript vector. Clones were selected randomly and sequenced with M13 M4 primer (Takara, Otsu, Japan) using a Dye Deoxy Terminator sequencing kit by DNA sequencer (373A; Applied Biosystems, Foster City, CA).

RESULTS

N-nucleotide insertion in Vγ1.1–Jγ4 transcripts during thymus development

To check the developmental regulation of N-nucleotide insertion in Vγ1.1–Jγ4 joints, we amplified cDNA of thymocytes at various developmental stages (day 16 of gestation, newborn and 7 weeks old) by PCR with Vγ1.1 and Cγ4 primers. About 30 DNA sequences were analysed for each sample (Table 1). The percentage of Vγ1.1–Jγ4 transcripts with a N-sequence in total transcripts was 3%, 42% and 89% in fetal, newborn and adult thymi, respectively. This result shows that N-nucleotide insertion at Vγ1.1–Jγ4 junctions in the thymus is developmentally regulated as previously reported with other TCR genes[2,14–16] and immunogloblin genes.[17,18]

Expression of TdT mRNA during thymus development

Because it has been shown that TdT plays a crucial role in N-nucleotide insertion,[19–21] we tested whether the level of TdT expression correlates with the level of N-nucleotide insertion in Vγ1.1–Jγ4 transcripts in the thymus. PCR amplification of serially diluted cDNA of thymocytes at three different stages was done with TdT and HPRT primers (Fig. 1a). Because the strength of the signals of HPRT decreased in proportion to the serial dilution of each template cDNA, the PCR reaction was considered to be done within the exponential phase. A significant level of TdT expression was observed in adult thymus, while those in fetal and newborn thymus were extremely weak. The intensity of the TdT signal was measured and revised by the intensity of HPRT signal. As shown in

Table 1. N-nucleotide insertion in Vγ1.1–Jγ4 transcripts derived from thymocytes

Stage of thymocytes	Total no. of transcripts	In frame transcripts (%)	N^+ transcripts (%)*		Added bases† per N^+ transcripts
			In total	In in-frame	
Fetal E16	30	44	3	8	2·0‡
Newborn	33	76	42	52	2·6 ± 0·35
Adult	27	44	89	83	2·9 ± 0·13

* The percentages of the transcripts with N-insertions in total and in in-frame transcripts are shown.

† The average numbers of added bases in the transcripts with N-insertions are shown as mean ± SEM. No statistical significance was observed by Student's *t*-tests.

‡ Fetal thymocytes included only one transcript with two bases of N-nucleotide insertion.

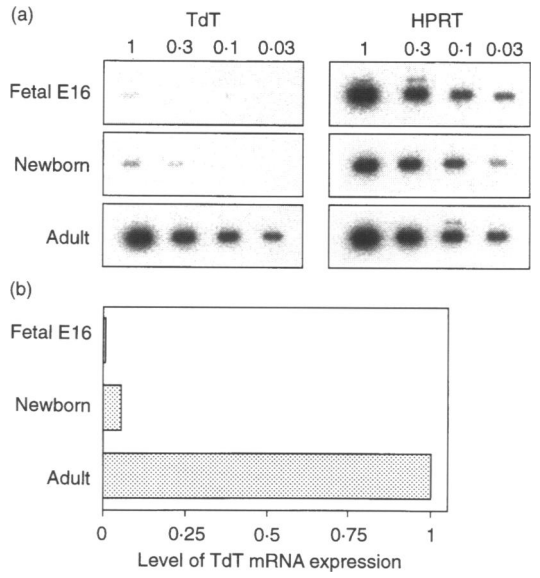

Figure 1. Expression of TdT mRNA in the thymus during ontogeny. (a) Quantitative PCR of TdT mRNA. cDNA was synthesized from thymocytes of day 16 fetuses (fetal E16), newborn or adult mice. Serial dilutions of cDNA were amplified with specific primers for TdT or HPRT, and the PCR products were analysed by Southern blot analysis. (b) Comparison of TdT mRNA expression during ontogeny. The intensity of the TdT and HPRT signals was quantified and the amount of each cDNA was revised by the level of HPRT expression. The ratio of the revised level of TdT is shown.

Fig. 1b, the relative level of TdT expression increased during ontogeny. It is noteworthy that there is a significant increase in TdT expression between newborn and adult stages. These data are basically consistent with the increase of N-nucleotide insertion in Vγ1.1–Jγ4 transcripts of thymocytes (Table 1). These data also support previous studies on TdT expression detected by immunofluorescence,[22] immunoprecipitation[23] and *in situ* hybridization.[24]

Junctional modifications of Vγ1.1–Jγ4 transcripts derived from IHL and spleen cells in normal and athymic nude mice

Flow cytometry analysis showed that γδ T cells in the liver of both normal and nude mice were approximately 5% of $CD3^+$ cells. In contrast, these cells in the spleen were about 1% of $CD3^+$ cells (data not shown). A previous report with other mouse strains supports our data.[10] The expression of Vγ gene was also analysed by PCR. Vγ1.1 and Vγ2 transcripts were detected predominantly in both IHL and spleen cells (data not shown).

To compare the junctional modifications, DNA sequences of Vγ1.1–Jγ4 junctions from IHL and spleen cells of normal and athymic nude mice were determined as shown in Fig. 2. In frame transcripts there were 87–89% of the total analysed clones. This percentage was higher than that of thymocytes (Tables 1 and 2). The percentage of N-nucleotide insertion in normal IHL and spleen was at intermediate levels (74% and 73%, respectively) between newborn and adult thymus. Eight out of 27 clones (30%) of normal IHL were common to the sequences of nude IHL (the common sequences were designated *a–d*; Fig. 2a, b). The percentage of N-nucleotide insertion of the common sequences was 38%, while that of the non-common sequences in normal IHL was 89%.

N-nucleotide insertion in nude IHL was at a similar level (47%) to that of newborn thymocytes (42%). Furthermore, about half of the sequences of nude IHL were common to those of newborn thymocytes (data not shown). These results imply that the Vγ1.1-expressing T-cell population in IHL of normal mice might consist of cells of extrathymic as well as intrathymic origins.

All the transcripts derived from nude spleens were with N-nucleotides. The majority of the sequences from nude spleen cells (91%) were common to those from normal spleen cells (the common sequences were designated *e–h*; Fig. 2c, d). These data suggest that Vγ1.1-bearing T cells with specific V–J junctions may populate in the spleen of normal and nude mice.

Variability of Vγ1.1–Jγ4 junctional sequences

We compared the variability of Vγ1.1–Jγ4 junctional sequences using the ratio of the number of species of DNA sequences to the total number of the sequences (Fig. 3). The variability scores of thymic cell sources elevated during mouse development. In adult thymus only six out of 27 clones appeared twice, while in fetal thymus 26 out of 30 clones repeated more than twice. This result suggests that the diversity of Vγ1.1–Jγ4 sequences in the thymus increases during development.

The variability scores of nude IHL and spleen cells were lower than those of normal IHL and spleen cells. It clearly suggests that the thymus plays an important role in increasing the diversity of Vγ1.1–Jγ4 junctions. Despite the high level of N-insertion, Vγ1.1–Jγ4 transcripts of nude spleen cells showed the lowest level of variability (Fig. 3). It is noteworthy that the

Table 2. N-nucleotide insertion in Vγ1.1–Jγ4 transcripts derived from IHL and spleen cells in normal and nude mice

Cells	Total no. of transcripts	In frame transcripts (%)	N^+ transcripts (%)		Added bases* per N^+ transcripts
			In total	In in-frame	
+/+ IHL	27	89	74	75	2·9 ± 0·27
+/+ Spleen	30	87	73	73	1·7 ± 0·32
nu/nu IHL	32	88	47	54	1·4 ± 0·26
nu/nu Spleen	35	89	100	100	2·4 ± 0·26

*The average numbers of added bases in the transcripts with N-insertions are shown as mean ± SEM.

(a)

Vγ1.1	N	Jγ4	Frequency	
GTC TGG ATA AA cac..		..gtg TCA GGC		
In frame				
TGG ATA		TCA GGC	4/24	*a*
TGG AT	GGG	A GGC	3/24	
TGG ATA	GCC	GGC	2/24	
TGG AT	GTCG	GGC	2/24	*b*
TGG AT	TGTC	TCA GGC	2/24	
TGG A		CA GGC	1/24	
TGG ATA AA T	AGGA	GC	1/24	
TGG A	AAC	CA GGC	1/24	
TGG	C	CA GGC	1/24	
TGG	G	GA TCA GGC	1/24	*c*
TGG AT	CCCT	GGC	1/24	
TGG A	C	A TCA GGC	1/24	
TGG AT	GGGG	TCA GGC	1/24	
TGG A	A	A TCA GGC	1/24	
TGG A		GA TCA GGC	1/24	
TGG	GGGG	GA TCA GGC	1/24	
Out of frame				
TGG	C	TCA GGC	1/3	
GT	AA	A GGC	1/3	
TGG A		TCA GGC	1/3	*d*

(b)

Vγ1.1	N	Jγ4	Frequency	
TGG ATA AA cac..		..gtg TCA GGC		
In frame				
TGG A	A	A GGC	9/28	*f*
TGG ATA		GGC	5/28	
TGG ATA		TCA GGC	5/28	*a*
TGG	G	GA TCA GGC	2/28	*c*
TGG ATA	C	GC	2/28	*e*
TGG		TCA GGC	2/28	
TGG	CCCG	GA TCA GGC	1/28	*g*
TGG AT	GTCG	GGC	1/28	*b*
TGG ATA AA TT		GC	1/28	
Out of frame				
TGG A		TCA GGC	3/4	*d*
TGG AT		GGC	1/4	

(c)

Vγ1.1	N	Jγ4	Frequency	
TGG ATA AA cac..		..gtg TCA GGC		
In frame				
TGG AT	G	GGC	5/26	
TGG ATA	C	GC	4/26	*e*
TGG A	A	A GGC	4/26	*f*
TGG ATA		TCA GGC	3/26	*a*
TGG ATA		GGC	3/26	
TGG	G	GA TCA GGC	2/26	*c*
TGG A	G	A GGC	1/26	
TGG ATA AA TT		GC	1/26	
TGG A	A	A TCA GGC	1/26	
TGG	GCCCTAC	CA GGC	1/26	
TGG	CCCG	GA TCA GGC	1/26	*g*
Out of frame				
TGG A	AAGG	GGC	1/4	*h*
TGG AT	TGC	GC	1/4	
TGG AT		GGC	1/4	
TGG AT	GGG	GGC	1/4	

(d)

Vγ1.1	N	Jγ4	Frequency	
TGG ATA AA cac..		..gtg TCA GGC		
In frame				
TGG A	A	A GGC	13/31	*f*
TGG	CCCG	GA TCA GGC	10/31	*g*
TGG ATA	C	GC	6/31	*e*
TGG	TTTC	GA TCA GGC	2/31	
Out of frame				
TGG A	AAGG	GGC	3/4	*h*
TGG	GAAGG	GGC	1/4	

Figure 2. Nucleotide sequences of Vγ1.1–Jγ4 junction from IHL and spleen cells of normal (+/+) and athymic nude mice (nu/nu). Junctional sequences from +/+ IHL (a), nu/nu IHL (b), +/+ spleen cells (c), and nu/nu spleen cells (d) are shown. The germ line sequences of Vγ1.1 and Jγ4 gene segments are shown in the top lines.[25,26] The nomenclature for mouse TCRγ chains is according to Garman *et al.*[27] N-nucleotides are shown in the middle column. P-nucleotides are underlined. The frequency of the particular sequence in analysed clones is listed. Common sequences among these junctions are shown as the letters *a–f* in the last column.

variability of spleen cells was lower than that of IHL in both normal and nude mice. These data also suggest that the Vγ1.1-bearing T cells in the spleen may represent a tissue-specific population.

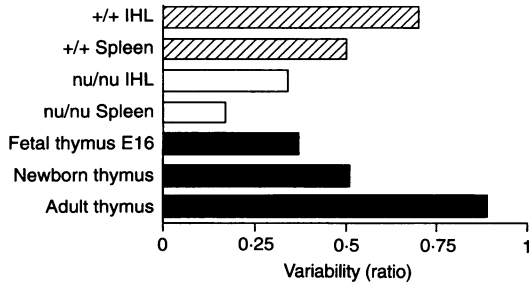

Figure 3. Variability of Vγ1.1–Jγ4 junctional sequences. Variability is shown as the ratio of the number of species of sequence divided by the number of total analysed clones.

P-nucleotide addition and base deletion at Vγ1.1–Jγ4 junctions

We next analysed the frequency of P nucleotide addition and the average of base deletion (Table 3). The percentage of transcripts with P-nucleotide addition in fetal thymus was remarkably lower than that of other cell sources. It was due to the high frequency of direct recombination via short sequence homology with P-nucleotide (Fig. 4). Because we referred to the stretch of homologous bases as germ line nucleotides, P-nucleotides did not exist at the joints by homology-directed recombination. When we counted them as P-nucleotides, not as germ line nucleotides, the percentage of transcripts with P-nucleotides in fetal thymus was calculated as 47%. Therefore, the frequency of P-nucleotide addition might not be different between all cell sources.

Average base deletion was from four to six nucleotides in all cell sources. In contrast to N-nucleotide insertion, the nibbling of coding ends seemed to be the same during development. The base deletion at the J5′ ends (1–2 nucleotides) was less than that

Table 3. P-nucleotide addition and base deletion in Vγ1.1–Jγ4 junctions

Cells	Percentage of P$^+$ transcripts*	Number of P$^+$ transcripts		Base deletion per transcripts†	Base deletion	
		V3′ end	J5′ end		V3′ end	J5′ end
+/+ IHL	22	1	5	4·5	3·4	1·1
+/+ Spleen	17	1	4	5·3	3·1	2·3
nu/nu IHL	13	1	3	4·8	3·2	1·6
nu/nu Spleen	34	0	12	5·8	4	1·8
Fetal thymus E16	3	0	1	4·2	3·3	1
Newborn thymus	36	2	10	4·7	2·9	1·7
Adult thymus	37	0	11	5·1	4	1·2

* The percentage of transcripts with P-nucleotide additions was calculated per the total number of clones.
† The average number of base deletions from the total number of transcripts was calculated.

at the V3′ ends (3–4 nt), suggesting that base deletions occurred more extensively at the V3′ end than at the J5′ end. Because base deletions occurred predominantly at the V3′ end, P nucleotide additions were more frequent at the opposite J5′ end.

V–J recombination with short-sequence homology

It has been reported that the recombination mediated by short sequence homology contributes to the generation of a limited diversity of V–(D)–J junction.[6,15,18,28] Recombination to short homologous regions in which P-nucleotides are possibly involved as an extension of the germ line sequence results in the absence of N-nucleotide addition.

In 214 clones studied, 83 transcripts displayed no N-nucleotide addition. In 45 transcripts, direct recombination via short-sequence homology was potentially considered. These transcripts were classified as five types, as shown in Fig. 4. The homology of type 1 contained three homologous nucleotides including two bases of P-nucleotide. The remaining four types contained only one base of germ line or P-nucleotide. However, it was difficult to determine whether the one nucleotide was responsible for the homology-directed recombination.[29] Therefore, we excluded the recombination by one-nucleotide homology from the following analysis.

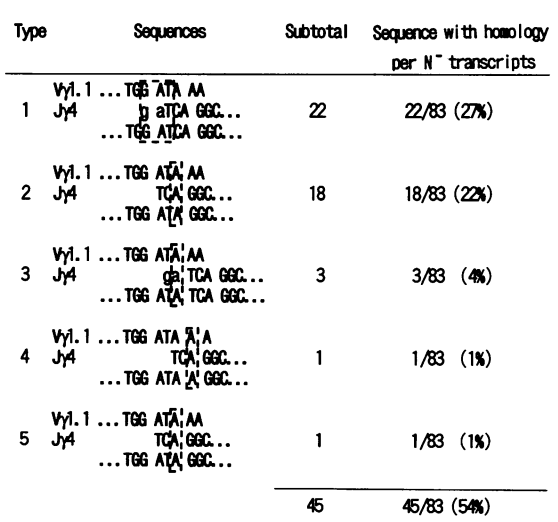

Figure 4. The short sequence homology at the Vγ1.1–Jγ4 junction. The germ line gene sequences of V and J segments are aligned on the basis of sequence homology, which are boxed. P-nucleotides, in lower letters, are included in the short homology. The frequency of the sequence with short homology is shown as the percentage in all transcripts without N-nucleotide additions analysed in this study.

The frequency of homology-mediated recombination in thymic cell sources is shown in Table 4. The frequency of short homology-directed recombination gradually decreased during the course of mouse development in the same way as the frequency of transcripts without N-nucleotide addition. Forty-three per cent of transcripts (13 out of 34) in fetal thymus showed direct recombination with short sequence homology involving P nucleotide.

The restricted diversity of the canonical sequences in Vγ3 and Vγ4 TCR is considered to be generated by homology-directed recombination.[15] The frequency of Vγ1.1–Jγ4 transcripts by homology-mediated recombination in fetal thymus (43%) was at a lower level than that of the Vγ3 and Vγ4 transcripts (76–80%) reported previously.[15] In addition the homology-mediated recombination at the Vγ1.1–Jγ4 junction was out of frame (Fig. 4).

The frequency of homology-directed recombination decreased in reverse to the level of TdT transcripts during mouse development (Fig. 1 and Table 4). This result was consistent with *in vitro* studies reported previously.[30]

Table 4. Short homology-directed recombination in Vγ1.1–Jγ4 junctions*

Stage of thymocytes	N$^+$ transcripts	N$^-$ transcripts	
		With homology	Without homology
Fetal E16	3	43	54
Newborn	42	15	43
Adult	89	0	11

* The percentage of N$^+$ transcripts and N$^-$ transcripts with or without homology is shown. The percentage of N$^+$ transcripts is the same as that shown in Table 1.

DISCUSSION

Although γδ T cells are a minor population in the liver and spleen of adult mice, certain numbers of γδ T cells were detected by flow cytometry analysis. Most transcripts of Vγ1.1–Jγ-Cγ4 junction were in-frame in IHL and the spleen cells, suggesting that functional Vγ1.1 TCR are expressed on some of the γδ T cells. It has been reported that over half of γδ T cells in the liver expressed Vδ6·3 or Vδ4 chains, and that Vγ1.1/Vδ6·3 TCR are frequently expressed on HSP-reactive hybridomas derived from IHL.[9] These data suggest that the in-frame Vγ1.1 transcripts may encode functional receptors in conjunction with Vδ6·3 or Vδ4.

γδ T cells with biased Vγ1.1–Jγ4 junctions may populate the spleen of nude and normal mice. It remains unclear whether specific T cells with rearranged receptors preferentially populated the spleen, or if T-cell precursors migrated and proceeded to rearrange TCRγ genes in the organ. A previous study suggested that the spleen is one of the extrathymic sites where the rearrangement of TCR genes takes place,[31] implying the possibility that specific clones may develop and expand *in situ*. However, the observation that rearranged γ genes were expressed in the early fetal liver in the absence of thymic influence suggests another possibility, that the fetal liver cells with rearranged TCRγ chains might directly populate the spleen of nude mice.[32] The common sequences between the liver and the spleen in nude mice were frequently detected (Fig. 2). These data are in favour of the second possibility, of the direct migration of fetal liver cells with rearranged TCR. γδ T cells in sheep expressing high levels of L-selectin showed preferential migration for peripheral lymph nodes.[33] The expression of organ-specific homing receptors could be a third possibility.

The transcripts derived from normal IHL were divided into two groups in terms of N-insertion level. One group, common with nude IHL, showed N-insertions at the newborn thymus level (38%). The other group was comparable with the adult thymus (89%). This suggested that these two groups of transcripts might be derived from different subpopulations of γδ T cells in IHL. These subpopulations might have developed at different stages of ontogeny, i.e. at newborn and adult stages. Because N-insertion in Vγ1.1–Jγ4 transcripts in nude IHL and spleen cells was at various levels (47% and 100%, respectively), it is not likely that all of the extrathymic microenvironment uniformly causes the same level of N-insertion. Rather it is possible that the N-insertion level in T cells of extrathymic origin is determined either by a property of T precursors or by extrathymic microenvironments. However, it remains unclear whether the Vγ1.1 T cells in nude IHL developed at around the newborn stage.

It has been reported that Vγ1.1/Vδ6 TCR bearing hybridomas produce cytokines spontaneously, and that the production requires the expression of the vitronectin receptor[34] or the recognition of mycobacterial HSP.[35,36] Some of the Vγ1.1–Jγ4 junctional sequences of our study were same as 'self-reactive' hybridomas (Fig. 2).[9,37,38] These data suggest that some Vγ1.1 T cells in IHL or spleen cells may interact with the extracellular matrix by vitronectin receptors or react with HSP 60, and produce various cytokines spontaneously.

It has been reported that the proportion of γδ T cells in the liver increased after birth and peaked at around 2 weeks.[9,37] These γδ T cells may develop at newborn or earlier stages, and their N-insertion level may be low. These data imply that γδ T cells expressing Vγ1.1 may play certain roles early after birth. It is possible that Vγ1.1-expressing T cells in the liver react at the newborn stage with various antigens delivered via the portal vein from the intestine.

ACKNOWLEDGMENTS

We thank Dr A. Mabuchi and Ms M. Kitajima (Nippon Medical School) for their technical assistance, and Dr M. Fujiwara (Faculty of Medicine, University of Tokyo) for encouragement. This study was supported by grants from the Ministry of Education, Science and Culture, Japan.

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